ECOLOGICAL OBSERVATIONS ON *AMBYSTOMA OPACUM*

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Several reports dealing with the life history of *Ambystoma opacum* Gravenhorst have appeared, among which are the works of Mann (1855), Deckert (1916), Dunn (1917), Brimley (1920), Bishop (1924), Lantz (1930), Noble and Brady (1930 and 1933). The species has been found in scattered areas over Ohio, but no previous studies concerning its breeding habits within the state have been published. In studying the ecology of certain upland forest areas in southwestern Ohio this species was encountered several times and its nesting sites located. The observations made to date seem to be of sufficient interest to justify this report. Physiological studies have been carried on in the laboratory as a means of further analyzing the conditions found in nature.

NESTING

Ambystoma opacum, in southwestern Ohio, inhabits what are known locally as "swamp forests." These forests are located on upland flats of impervious soil from which the water drains very slowly. The forests differ in their degree of development from the pin-oak to a nearly pure beech forest. Combinations of white elm, red maple, hickories and gums are found in the different stands. The herbaceous story is limited. Tree roots crowd to the surface, indicating a soil that is saturated with water a considerable portion of the year. In this type of environment I have found this salamander in Clinton county near Villar's Chapel, in Warren county along the earthworks at Fort Ancient and in an oak-hickory forest in the southwestern corner of the county, and in Clermont county near Goshen Station.

Adult salamanders are found most commonly in the months of September and October. The species is rarely met with at other times. Clues which led to the discovery of the nests were the finding of the larvae in certain pools in the spring of 1932 and 1933, and the observations of Noble and Brady

(1933). These authors state that "*Ambystoma opacum* lays its eggs under leaf mold, sphagnum, dry water weed or other cover in situations which will be flooded by the winter rains." Mann made the first observations on the breeding habits of this species at Gloucester C. H., Virginia, in 1854. He wrote the following description: "The localities are beds of small ponds in the woods, which in rainy seasons have water in them. . . . The nests . . . were in a small hollow in the surface of the earth, deeply covered with leaves, and under which were tunnels extending in all directions. In these hollows the animals were . . . curled up over their eggs." Not all parts of the wooded areas offer suitable breeding grounds, but only certain depressions, either natural or artificial, are chosen for the nests. Only one nest was found under an inverted half of a hollow log. All others were beneath leaf litter. Mouse or crayfish tunnels are utilized, but many nests show no connection with other excavations and must be made by the female salamander. The male has not been observed to have any part in nest building. The nests are readily found by turning the over-lying litter with a small hand rake. They measure three to four inches in diameter and are from one to two inches deep. The nests are usually not in the dampest part of the depression, but along the margins of the low banks or on a raised portion of the pool floor. Adequate cover is essential as a concentration of nests in the most favorable places shows. Some moisture is required, but as shown below too much water favors premature hatching.

The time of egg laying was not exactly determined, but chiefly occurred between September 27 and October 5. This was ascertained from the development of eggs laid in the laboratory by a female salamander collected October 6. This animal was placed in a box containing moist sand covered with moss. She hollowed out a small area beneath the moss and laid 81 eggs on October 8. Six days were required to reach the degree of development possessed by eggs collected October 10. With few exceptions all the eggs collected did not vary more than five days in their degree of development. Twenty-five nests were observed and counted. The average number of eggs was 98 per nest. Egg counts of individual nests were 120, 91, 85, 90, 80, 69, 155, 112, 87, 147, 73, 104, 89, 116, 69, 150, 116, 99, and 99. There was also one double nest with 174 eggs and a combination of four nests which averaged

84 eggs each. A second counting, 66 days after the first, was made on four nests in the field.

Nest Number	1	2	3	4
Number of eggs, October 13.......	147	69	87	155
Number of eggs, December 18.....	141	53	70	134

The average loss was slightly over 10%. Eggs which have failed to develop can be recognized by their discoloration. These were not included in the second counting. A number of the eggs are no doubt destroyed by beetles and centipedes which are common visitors in the nests.

In agreement with earlier writers and contrary to Noble and Brady (1933), I have always found the female on top of the eggs. Her tail may be curled or straight, but her body invariably rests on the egg clutch. The accompanying photograph shows this clearly. Variation in the length of time that the female stays with the eggs has been found. When the females are separated from the nests during the counting of the eggs they usually lie quietly on the leaf litter, making no effort to escape. After being returned to their nests they may or may not continue to brood the eggs. Four of seven females were found to be still brooding their nests after a temporary removal. One female was disturbed for photographing twice but stayed with her nest two weeks longer. Females were brooding undisturbed nests November 6, about one month after egg laying, but had left a month later, December 6. All females whose nests were disturbed left before November 6. The eggs have a coating of soil adhering to them. Since not all are in contact with the soil at one time it appears that the female must burrow through the eggs, changing their position. Her body is free from dirt. In deserted nests many of the eggs become matted together. Such clumps have a low percentage of development. Males are occasionally found in the nesting area after egg laying, but are usually scattered in the vicinity of the nesting grounds.

HATCHING

It was found that the growth of the embryo follows very closely the growth stages of *Ambystoma maculatum* as figured by R. G. Harrison (1925). Consequently the "Harrison

Stages" are used in recording the early growth of *Ambystoma opacum* larvae. Laboratory experiments were conducted to determine the percentage of eggs capable of hatching and the ages at which hatching occurs. From a total of 220 eggs immersed in water, 182, or 80% (Table I) hatched.

It was found that it is not always necessary for an egg to be immersed in water in order to hatch. After heavy rains, insufficient to flood the nest but moistening it, some larvae will hatch. This was observed in the field December 18 and again January 8. The larvae emerging under these conditions perish if the rainfall is insufficient to provide permanent inundation.

TABLE I

HATCHING EXPERIMENTS

All larvae except those in nest 3 were placed in fingerbowls in tap water in the laboratory. The eggs in number 3 rested in a depression made in tightly packed clay. These eggs were kept outdoors. Rains covered the eggs to a depth of one-half inch between October 27 and 30. Eighty of these 100 eggs hatched during the three days of exposure. Five more hatched during the following week.

NUMBER OF THE NEST	1	2	3	4	5	6
Number of eggs	12	25	100	25	48	10
Number of larvae	11	19	85	13	48	9
Total days immersed	10	2	3	5	½	2
Harrison stage at hatching	42–3	42–4	44–6	42–6	....	....
Total length, mm	13	13	13–5	13–5	15–8	18
Temperature, degrees C	21	21	15	21	21	21
Date of hatching	Oct.20	Oct.20	Oct.27	Oct.30	Nov.23	Dec. 9

If the nests were located in the dampest part of the area, the first heavy rain would enable all the larvae to hatch. Subsequent drying would be fatal to the entire population. The habit of placing the nests beneath heavy leaf litter and at a slight elevation usually prevents such premature hatching, since most of the eggs do not hatch before permanent flooding occurs. The completion of hatching is attributed to the action of unicellular glands on the head, but in addition water must be absorbed before hatching can occur. The softening of the envelopes plus the wriggling movements of the larvae permit its escape. Either the head or the tail may emerge first. Larvae with the gelatin capsules still fastened over their heads are common sights.

The embryo is able to emerge from the gelatin by the time it has reached Harrison stage 42. Noble found larvae able to hatch in a slightly less developed condition, stage 38 approximately. Two-thirds of the larvae used in the same experiment, however, hatched at a later stage comparable to the above. Eggs kept at low temperatures until past their normal hatching time were able to emerge when as young as stage 40. This

TABLE II

GROWTH OF LARVAE FROM EGGS LAID IN THE LABORATORY

Eighty-one eggs were laid in the laboratory October 8. October 10 only 11 showed embryological development. These were placed in a fingerbowl containing tap water at room temperature, and daily observations made.

DATE	HARRISON STAGE	TOTAL LENGTH, MM.	REMARKS
October 10	8–9	3 (D. of capsule)	11 living
October 11	21		11 living
October 12	27	4 (D. of capsule)	11 living.
October 13	31		8 living.
October 14	35		7 living.
October 16	37	5 (D. of capsule)	1 photographed.
October 18	39		
October 20	40		
October 23	42	12 total length	6 living.
October 27	43	12.5 total length	5 hatched Oct. 28–9.
November 3	46	17 total length	pH of water 7.9.
November 10	46 plus	18 total length	5 survivors.
November 17	46 plus	18 total length	4th digit appeared.

TABLE III

GROWTH OF LARVAE SECURED FROM FORT ANCIENT

DATE	HARRISON STAGE	TOTAL LENGTH, MM.	REMARKS
October 10	35	8	These larvae were freed from the egg capsules at the beginning of the experiment. Six larvae were used. The water was kept at room temperature. Its pH was 7.9.
October 12	37	10.5	
October 14	40	13	
October 16	41	15	
October 18	44	16	
October 20	46	18	
October 23	46 plus	18	
October 27	46 plus	19	
November 3	46 plus	20	

degree of development may be accomplished in 15 days or may require two months, depending on the temperature. In order to learn the lowest temperature at which eggs of *Ambystoma opacum* might hatch, five sets of ten eggs each were placed in water at 21° C., 7°, 5°, and 2° C. After 12 hours immersion ten larvae had emerged from the eggs kept at 21° C., five from the set at 12°, two from the set at 7°, two from the set at 5° C., none from the set at 2° C. Eggs were often kept in water in the Frigidaire at a temperature of 2° to 3° C. without hatching. Thus 5° C. appears to be the minimum temperature at which the eggs will hatch. Eggs from which the outer capsules have been removed often fail to hatch even though placed in a favorable medium. The remaining capsule swells decidedly, becoming even larger than the original complete egg. The digestive action of the glands is reduced so that hatching is delayed.

THE EFFECT OF THE ENVIRONMENT ON GROWTH

It is apparent that the gelatin capsules are a vital necessity for the early life of the embryo. If the eggs are immersed in water before they are sufficiently developed to hatch, the ability to hatch appears lessened. However, embryos as little advanced as Harrison stage 35 thrive better without their capsules. Larvae of this stage are able to swim actively upon mechanical release. Larvae described in Table III were 18 mm. in length and were in stage 46 on October 20, ten days after having been freed from their capsules. Eggs from the same collection and kept in the same environment with capsules intact hatched October 20. These larvae had attained a total length of 13 mm. and were in Harrison stage 42–3. In another experiment liberated larvae measured 20 mm. the day that eggs from the same nest and kept in the same fingerbowl hatched at a total length of 17 mm. With all other factors remaining constant, larvae that have been freed from their capsules after having reached stage 35 show a more rapid development than those with capsules intact.

In view of these observations it seemed that rapidity of growth might also be dependent upon a number of factors such as temperature, ability to move about and the food available after hatching. A Frigidaire and a laboratory room offered uniform environments in which to conduct experiments necessary to show the effects of these different conditions, (Table IV). Eggs from the same nest were used. All larvae showed a similar degree of development, Harrison stage 40, at the beginning of the experiment. Three groups of animals were used in each location. All were kept in the dark. In Experiment A, eggs with capsules intact were placed on damp soil. In B, eggs with capsules intact were placed in water. In C, larvae freed from their capsules were placed in water. The experiment was

TABLE IV

EXPERIMENTS SHOWING THE EFFECTS OF ENVIRONMENTAL FACTORS ON GROWTH. DURATION OF EXPERIMENT, 30 DAYS

ORIGINAL CONDITION OF LARVAE IN EXPERIMENTS A, B, AND C	EXPERIMENT A_1, FRIGIDAIRE TEMPERATURE 2° C.	EXPERIMENT A_2, LABORATORY TEMPERATURE 21° C.
Diameter of entire egg, 6 mm. Total length of larvae, 14–14.5 mm. Harrison stage 40. Fore limb bud undivided. No hind limb buds. Gills with few rami. Balancer present. Considerable yolk present.	10 eggs on water saturated soil. 6 survived, none hatched.	10 eggs on water saturated soil. 2 survived in the egg. 4 hatched on damp soil, perished. 4 perished in the egg.
HABITAT CONDITIONS, EXPERIMENT A	Development: Total length, 14–14.5 mm. Harrison stage 40–41. Fore limb bud undivided. No hind limb buds. Gills with few rami. Balancer present. Little change in yolk content.	Development: Total length, 16 mm. Beyond Harrison stage 46. 4 digits on fore limb. Hind limb buds microscopic. Gill rami well developed. No balancers. More pigmented than A_1. Yolk practically exhausted.
Pint glass jars used as containers. Kept closed. Air practically saturated with water vapor. pH of soil in A_1, 5.6. pH of soil in A_2, 6.2.		
EXPERIMENT B, WATER ANALYSIS	EXPERIMENT B_1, FRIGIDAIRE	EXPERIMENT B_2, LABORATORY
(Frigidaire / Laboratory) pH .... 7.2 / 7.5 O_2, cc. per liter ..... 9.00 / 5.82 Free CO_2, per liter .. 3.03 / 3.06 Fixed and half-bound CO_2 per liter ...... 8.00 / 9.80	10 eggs entire, in 250 cc. tap water. 9 survived, none hatched. Development: Total length, 15 mm. Harrison stage 40–41. Fore limb buds undivided. No hind limb buds. Gill rami small. Balancers present. Possibly less yolk than in A_1.	5 eggs in 250 cc. tap water. 4 hatched within 24 hours. 3 larvae survived. 1 perished in the egg. Development: Total length, 18 mm. More advanced than Harrison stage 46. 4 digits on the fore limb. Hind limb buds microscopic. Gill rami well developed. No balancers. No yolk material remaining. Sise of head large in relation to body length, typical of starved larvae.

Water analysis, Experiment B:

	Frigidaire	Laboratory
pH	7.2	7.5
O_2, cc. per liter	9.00	5.82
Free CO_2, per liter	3.03	3.06
Fixed and half-bound CO_2 per liter	8.00	9.80

TABLE IV—Continued

EXPERIMENTS SHOWING THE EFFECTS OF ENVIRONMENTAL FACTORS ON GROWTH. DURATION OF EXPERIMENT, 30 DAYS

EXPERIMENT C, WATER ANALYSIS	EXPERIMENT C_1, FRIGIDAIRE TEMPERATURE 2° C.	EXPERIMENT C_2, LABORATORY TEMPERATURE 21° C.
Frigidaire / Laboratory pH.................. 6.9 / 7.05 O_2, cc. per liter..... 7.79 / 4.49 Free CO_2 per liter... 4.20 / 3.03 Fixed and half-bound CO_2 per liter...... 7.59 / 5.09	10 larvae freed from their capsules. Kept in 250 cc. tap water. 9 survived. Development: Almost identical to that in B_1. Total length, 15 mm. Harrison stage 40–41. Fore limb buds undivided. No hind limb buds. Gill rami small. Balancers present. Less yolk in gut than in B_1. Larvae lay inactively on the bottom most of the time.	10 larvae freed from their capsules. Kept in 3000 cc. tap water. 10 larvae survived. Development: Total length, 19–20 mm. Considerably more advanced than Harrison stage 46. 4 digits on fore limb. Hind limb bud microscopic. Gill rami well developed. No balancers. Gut filled with food. Larvae active. Their gills show some evidence of cannibalism. These larvae the most advanced of all experimentals.

conducted for 30 days, at the end of which time all larvae were examined and measurements taken. In general, it was found that none of the animals kept in the Frigidaire showed appreciable development. A slight advantage might be accredited to group C over the others, but even this is too small to be significant. Marked differences were seen in the animals kept in the laboratory. In Experiment A_2, 40% of the larvae hatched on the wet soil. At no time were the eggs covered with water. The two survivors show a significantly greater growth than those kept in the colder temperature but with other factors constant. In Experiment B_2, where the larvae hatched almost immediately, the effect of temperature on the ability to hatch is seen. Eggs kept at 2° C. did not hatch. Also since no food was present the extent of growth was limited, although freedom of movement was possible. In Experiment C_2, the most favorable situation regarding temperature, food and freedom of movement were found. A larger quantity of water was necessary to prevent mutilation of gills and appendages by other larvae. These larvae outstripped all others in their growth, reaching a total length of 20 mm., with the development of appendages and other organs in proportion. The difference in development shown by the two groups of animals in Experiment B is influenced by the fact that in B_2 the larvae hatched almost immediately. That this increased growth was not due to freedom of movement alone is shown in Experiment B_1 and C_1 by the failure of larvae freed from their capsules to grow appreciably more than those confined within the egg membranes, kept at the same temperature and in the same volume of water. While the results of this experiment show no new phenomena, quantitative effects of such known factors as freedom of movement, temperature, presence of water and food in their relation to growth are definitely shown.

At the time of writing, February 5, 1934, conditions in the field are severe in respect to the survival of the eggs and larvae of *Ambystoma opacum*. At two breeding sites under observation partial flooding occurred January 5 and 8, so that apparently all the eggs hatched. Within the month all the water either froze solidly or evaporated, causing the death of the larvae. Some pools are still dry, and here a good proportion of the eggs are yet viable. Their fate depends upon sufficiently heavy and permanent rains in the weeks that follow.

SUMMARY

1. *Ambystoma opacum* is an inhabitant of the swamp forests of southwestern Ohio.

2. Its nests are located on the margins of depressions which are pools approximately six months of the year.

3. The average number of eggs in 25 nests was 98.

4. The females brood the nests for at least a month. They were always found with their bodies resting upon the eggs.

5. The early growth is very similar to that of *Ambystoma maculatum* and may be compared with the Harrison stages figured for that species.

6. While a definite stage must be reached before hatching can occur, this growth may be accomplished in as short a time as 15 days. Older larvae hatch at a slightly less advanced condition than younger larvae that have grown more rapidly.

7. Eggs were not able to hatch at 2° C., but four out of twenty hatched at 5° C. The highest percent hatched at room temperature, 21° C.

8. Embryos of Harrison stage 35, 8 mm. in length, removed from their egg capsules and placed in water grow more rapidly than those that have been retained within the capsules.

9. A low temperature, 2° C., and dessication retard the growth of the larvae, while freedom of movement at a higher temperature, 21° C., and presence of food, Copepods and Ostracods, favor their growth.

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EXPLANATION OF PLATE

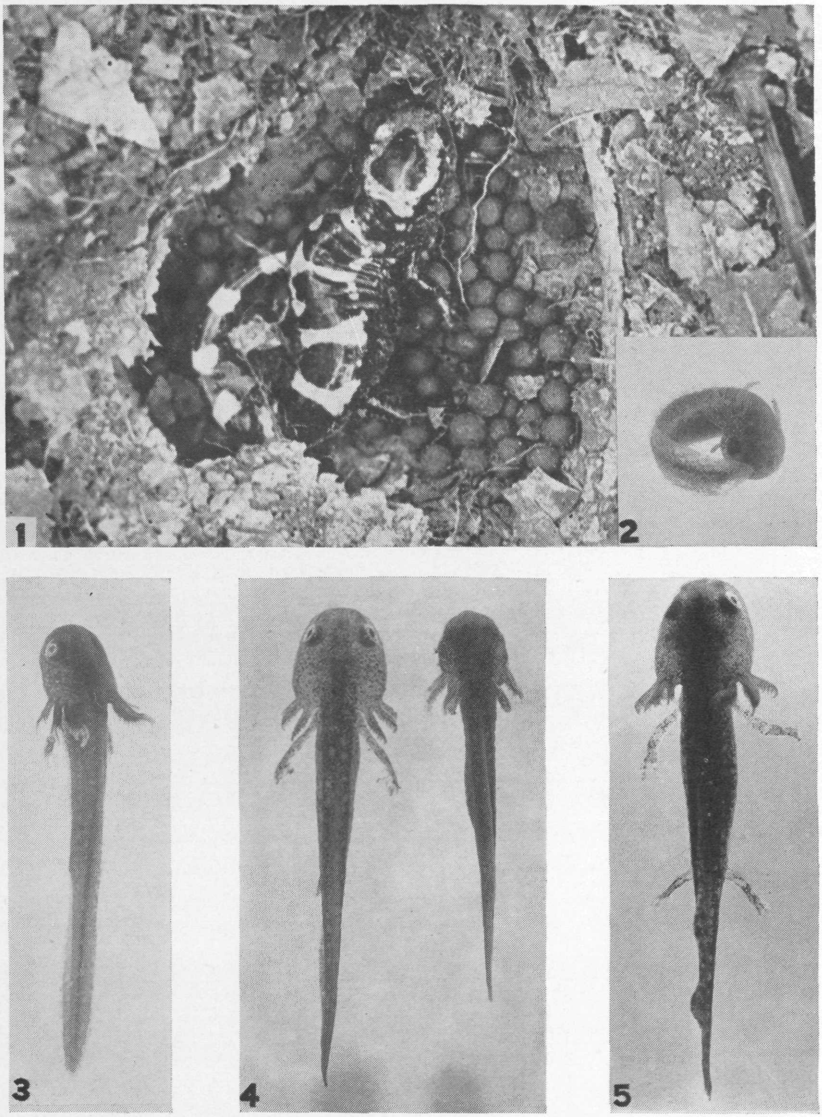
Fig. 1. Female *Ambystoma opacum* brooding her nest. Warren County, October 21, 1933.

Fig. 2. Egg with outer capsules removed. Harrison stage 40, 8 mm. total length. 5 × natural size.

Fig. 3. A typical hatching stage, Harrison stage 45. Total length, 18 mm. Larvae from Fort Ancient, December 15, 1933. 5 × natural size.

Fig. 4. Experimental animals showing the effect of temperature on growth. The larger animal was kept at 21° C., the smaller at 2° C. Both were taken from the same nest and freed from their capsules at the beginning of the experiment. The larger larvae shows four digits on the fore limb, rudimentary hind limbs, no balancer present, gill rami well developed. The smaller one has the fore limb bud undivided, no hind limb bud, balancer present, gill rami small. Similar food was available to both animals. All other larvae of the same sets are identical with these figured. 5 × natural size.

Fig. 5. A more advanced larvae, kept in an aquarium at room temperature for two months after hatching. 4 × natural size.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